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Hydrocortisone biotransformation pathway in three types of river-based aquifers media and changes in microbial community

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ABSTRACT

The biodegradation characteristics and primary metabolic pathway of hydrocortisone were investigated in different aquifer media-water systems. The aquifer media from Beijing Chaobai river (BJ), Hebei Hutuo river (HB), and Tianjin Duliujian river (TJ), having different properties were used. The results showed that the attenuation rates of hydrocortisone were 0.175, 0.119 and 0.096 d^{-1} in BJ, HB, TJ respectively and all of them followed the first-order rate kinetics. The main metabolic pathway of hydrocortisone in the BJ system began with the hydration of C-4 and C-5 double bond on ring A, and the A-ring was subsequently cleaved. In the case of the HB system, the primary biotransformation pathway started from the side chain degradation and oxidation of C-12 on ring D to form a ketone group, and the D-ring was subsequently carboxylated and cleaved. The differences in attenuation rates and metabolic pathways were associated with community structures of bacteria and archaea in different systems. The biodegradation of A-ring had a greater influence on the decay rate than D-ring. The results from microbial community structure analysis using 454 pyrosequencing of 16s rRNA genes showed that the microbial community structure transformed to microflora with good degrading capacity towards hydrocortisone by hydrocortisone amendment. The potential bio-transformers were seven major bacterial genera (*Methylophilus*, *Sphingopyxis*, *Arenimonas*, *hgcI_clade*, *Methylobacillus*, *Methylotenera* and *Fluviicola*) and two major archaeal genera (*Candidatus_Nitrososphaera and Woesearchaeota_DHVEG-6*), respectively.

1. Introduction

The effluent from wastewater treatment plants and reclaimed water are considered as the primary river's ecological base flow in several arid areas of China. This might be an effective approach to restore the river and lake ecosystems (Sun et al., 2016a; Ma et al., 2018). This type of water accounts for more than 75% of the river's ecological base flow in several arid areas of China. In Beijing, a large amount of reclaimed water is being used as a supplementation for rivers and lakes in arid and semi-arid areas, for river basins like the Jian River and the Chaobai River (He et al., 2017). According to a recent government report, the amount of reclaimed water reuse for the restoration of river ecosystem has reached 7 billion m^3, accounting for 75% of the total water use in Beijing (Ministry of Environmental Protection of the People's Republic of China, 2017).

Wastewater effluent and reclaimed water reuse in ecological restoration of river and lake have been considered as a useful approach in order to ameliorate the serious water shortage prevailing in China, specifically in the case of dried rivers (Li et al., 2013a; Ma et al., 2015a, 2018). However, wastewater effluent and reclaimed water contains toxic and harmful organic matters, as glucocorticoids including hydrocortisone and cortisone. Glucocorticoids are commonly used endocrine disrupting hormone-like chemicals, which have many functions such as anti-inflammatory, antiviral, anti-allergic, anti-shock, non-specific immunosuppressive and antipyretic properties (Jia et al., 2016). In a recent study, it was shown that reclaimed water is a potential threat to river-based aquifers because endocrine disrupting chemicals (EDCs) accounted for ~27% of the priority organic compounds identified in reclaimed wastewater discharging areas in China (Li et al., 2014). The detection of hydrocortisone acetate in Qinghe river of Beijing was > 80% with an average concentration of 476 $ng.L^{-1}$ (Chang et al., 2007; Jia et al., 2016). According to recent literature, the concentration of hydrocortisone in Yongding river of Beijing and Drainage River of South of Tianjin ranged from 4.96 to 8.32 $ng.L^{-1}$, respectively (Guo et al., 2015; Ma et al., 2018).

Due to their hydrophobic properties, glucocorticoids are likely to

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Table 1
Physical and chemical properties of the representative soils from the vadose zone media.

Medium category	Soil particle size distribution (%)			Pore volume ($cm^3.g^{-1}$)	TOC (%)	Specific surface area ($m^2.g^{-1}$)	CEC ($cmol.kg^{-1}$)
	2–0.05 mm	0.05–0.002 mm	< 0.002 mm				
BJ	48.21	41.63	10.16	0.075642	2.09	27.60	13.55
HB	52.36	38.67	8.97	0.043493	1.78	26.84	11.27
TJ	39.65	52.67	7.68	0.011798	0.98	5.85	15.68

accumulate in the organic portion of the river-based aquifer media, resulting in very high concentrations in the aquifers. However, this accumulation could become the source of secondary pollution and it has strong negative impact on various aquatic microorganisms, groundwater quality, and even the food web (Ma et al., 2015b). Due to the presence of glucocorticoids, having high level of toxicity even at low concentrations, more attention should be paid to determine the pathway and toxicity of its metabolites under *in situ* natural attenuation in these rivers using reclaimed wastewater as receptors.

Beijing (BJ), Tianjin (TJ) and Heibei (HB) are typical water shortage areas in China, and each of them has its own unique physico-chemical properties and microbial community structure in the respective river-based aquifer medium. These three river-based aquifer media have total organic content (TOC), cation exchange capacity (CEC), and other physico-chemical properties, which not only affects the adsorption and desorption processes of organic contaminants in sediments, but they also control the natural attenuation rate and the metabolic pathway of hydrocortisones in the environment (Ghasemi et al., 2007; Dodgen et al., 2014). The microbial community structures in the three types of sediments also differ from each other. The aquifer media harboring diverse groups of microorganisms has good potential to remove EDCs, and microbial community changes during the removal could reflect the impact of EDCs on microorganism in the aquifer media (Al-Ansari et al., 2010; Ma et al., 2015a, 2018; Klute, 1986).

The potential of sewage treatment plants to remove glucocorticoids has been demonstrated, but it is difficult to make a direct comparison among the different river-based aquifers media because of the differences in aquifer medium type or due to the fact that their removal potentials are directly related to their microbial community compositions (Chang et al., 2007; Murphy, 2016). A few studies have reported that the degradation pathway of EDCs starts from the A-ring or D-ring (Li et al., 2013b; Ren et al., 2007; Chang et al., 2007), but further biodegradation pathway was rather difficult to propose or identify in soil-water systems (Murphy, 2016). The metabolic pathway of hydrocortisone degradation has been reported for single strains, such as *Nostoc muscorum* (Yazdi et al., 2004).

Ghasemi et al. (2007) reported side-chain cleavage and C-20 ketone reduction of hydrocortisone by a natural isolate of *Chroococcus* disperses. Many microbes have the ability to degrade endocrine disruptors, such as *Sphingbacterium*, *Rhodococcus*, *Candidatus_Nitrososphaera* and *Nitrosomonas* (Yoshimoto et al., 2004; Shi et al., 2002; Ren et al., 2007; Aris et al., 2014; Ma et al., 2018). Some of these studies have reported the changes in the microbial community structure under the influence of EDCs pollution, but they mainly focused on the sludge in sewage treatment plants with minimal attention given to study their effects on the different types of river-based aquifers media.

The discrepancy in the biodegradation rate and the metabolic pathway was caused by the difference in microbial community structure and diversity. Therefore, research pertaining to identify the relationship between the biotransformation pathway of hydrocortisone and microbial community structure is particularly important for assessing the potential environmental risk and the intrinsic degradation ability of aquifer medium to glucocorticoid. Therefore, the present study, based on a microcosm experiment, aims to propose possible metabolic pathways and evaluate changes in microbial community in three types of aquatic sediments, namely BJ, HB, and TJ, respectively. This study also identifies the potential functional microbial group involved in the removal of hydrocortisone. The results of this study will help to understand the behavior and fate of hydrocortisone and provide solutions for *in situ* river-based groundwater recharge with reclaimed water.

2. Materials and methods

2.1. Vadose zone soil sample collection and its physico-chemical properties

Simulation aquifer media were sampled from representative soil vadose zone media in the Chaobai River (39°48′N, 116°21′E), Duliujian River (39°05′N, 117°05′E) and Hutuohe River (38°02′N, 114°27′E), respectively, which is a typical sandy clay, fluvio-aquatic soil, and fine sand from North China. The respective aquifers media were collected from the sites by drilling suitable holes according to the profile of the upper dune sediments under the three rivers. Table 1 shows the physical and chemical properties of the representative soil vadose zone media. The bulk density ($mg.dm^{-3}$) was determined using the core method, while the cation exchange capacity (CEC) of the soil samples were measured according to the ammonium acetate method (Klute, 1986). Soil organic carbon (C) and total nitrogen (TN) were analyzed using dry combustion with a CHN elemental analyzer (Costech Analytical Technologies, Inc., Valencia, CA). The Autosorb-iQ-C (Quantachrome, Boynton Beach, FL, USA) was used to determine the specific surface area (SSA) of the soil and biochar samples.

2.2. Biodegradation potential of hydrocortisone

A microcosm biodegradation experiment was conducted using 350 mL quick-fit conical flasks, each containing 40 g of fresh aquifer media and 200 mL of reclaimed water to simulate the continuous infiltration mode by reclaimed water in rivers. The treatments were made as follows: (i) hydrocortisone-treatment: reclaimed water spiked with 500 $ng.L^{-1}$ hydrocortisone and aquifer media; (ii) sterilized control: the same as (i) and spiked with 200 $mg.L^{-1}$ sodium azide; and (iii) control: reclaimed water spiked only with aquifer media. The water used in this study was collected from the tertiary effluent of Yinwenjichao reclaimed water treatment plant (N 40.12°, E 116.50°), located in Beijing (China). The various unit processes of this reclaimed water treatment plant constituted of ozone pre-oxidation, a membrane bioreactor, chemical phosphorus removal, and disinfection. The main characteristics of the reclaimed water used for river-based groundwater recharge are as follows: NH_4^+ 1.0 $mg.L^{-1}$, TN 6.7 $mg.L^{-1}$, Na^+ 100.0 $mg.L^{-1}$, K^+ 18.0 $mg.L^{-1}$, Ca^{2+} 63.5 $mg.L^{-1}$, Mg^{2+} 24.0 $mg.L^{-1}$, Cl^- 93.3 $mg.L^{-1}$, SO_4^{2-} 69.0 $mg.L^{-1}$, and HCO_3^- 317.0 $mg.L^{-1}$. The water sample for this study was collected and stored in dark for a maximum of 5 d. The desired concentration of hydrocortisone (500 $ng.L^{-1}$) in the reclaimed water was obtained by adding pure hydrocortisone. Hydrocortisone (99.9% purity) was purchased from Sigma-Aldrich (USA). Ten replicates per treatment were spiked with hydrocortisone. All flasks were incubated under agitated conditions (150 rpm), at 25 °C for 50 d. Sediment slurries from the hydrocortisone-treatment (4 mL) and the sterilized control were collected on days 0, 2, 4, 6, 10, 14, 20, 30, 40 and 50 d, respectively, for hydrocortisone analysis.

The total attenuation was calculated as follows: